Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Tracking the effects of PLGA-based nanoparticles on protein expression in living cells through quantitative proteomics

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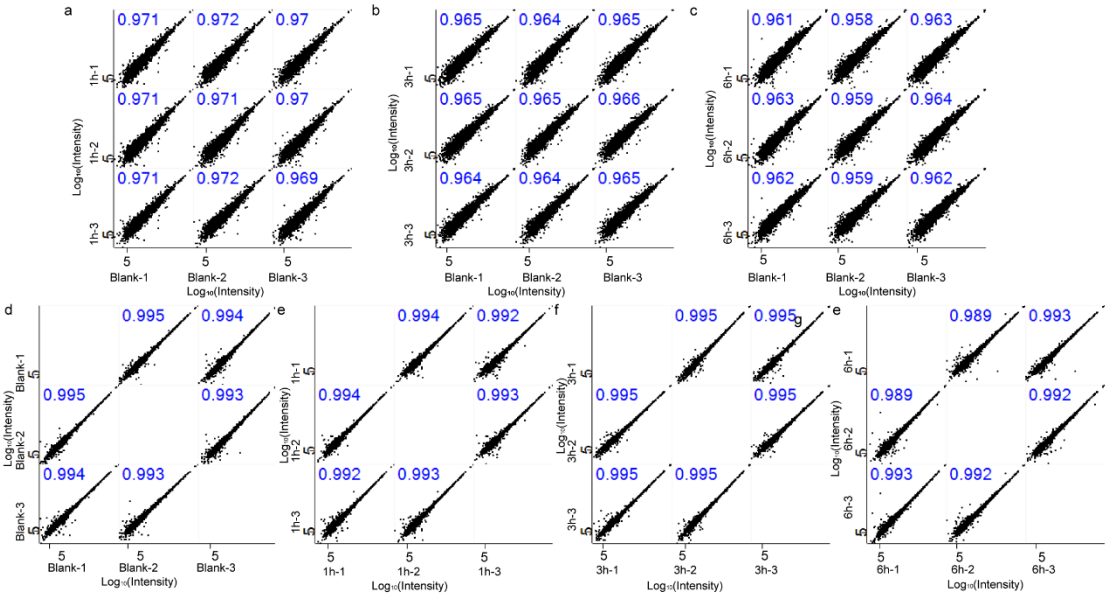
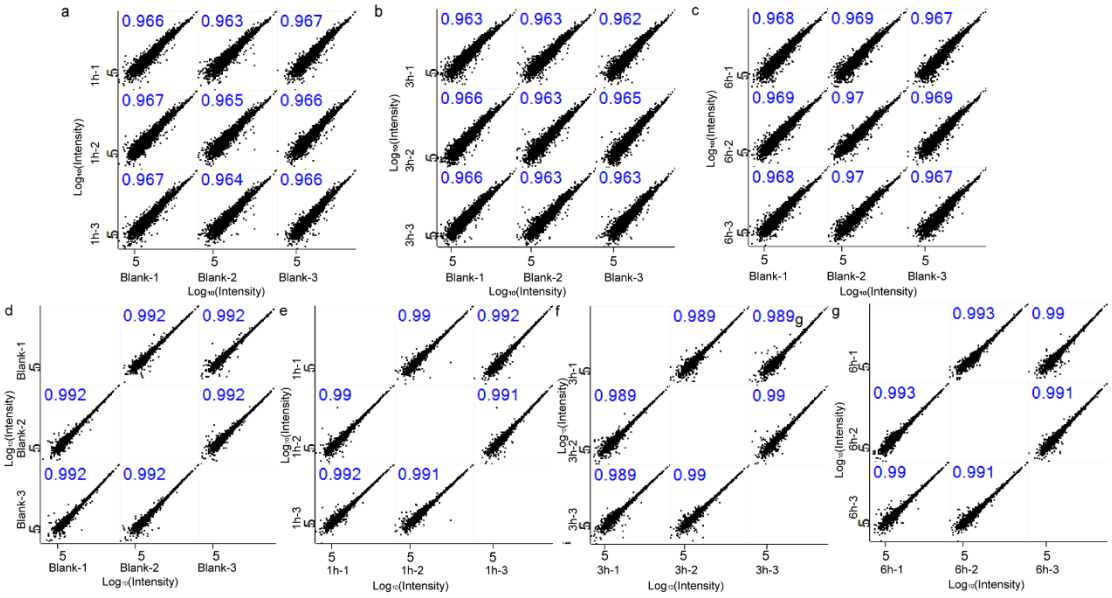
Supplementary Figures and Table

Supplementary Table 1: The loading efficiency of DSS in the 10DSS-DDAB@PLGA/Kolliphor EL NPs, 40DSS-DDAB@PLGA/Kolliphor EL NPs and 70DSS-DDAB@PLGA/Kolliphor EL NPs, measured using HPLC.

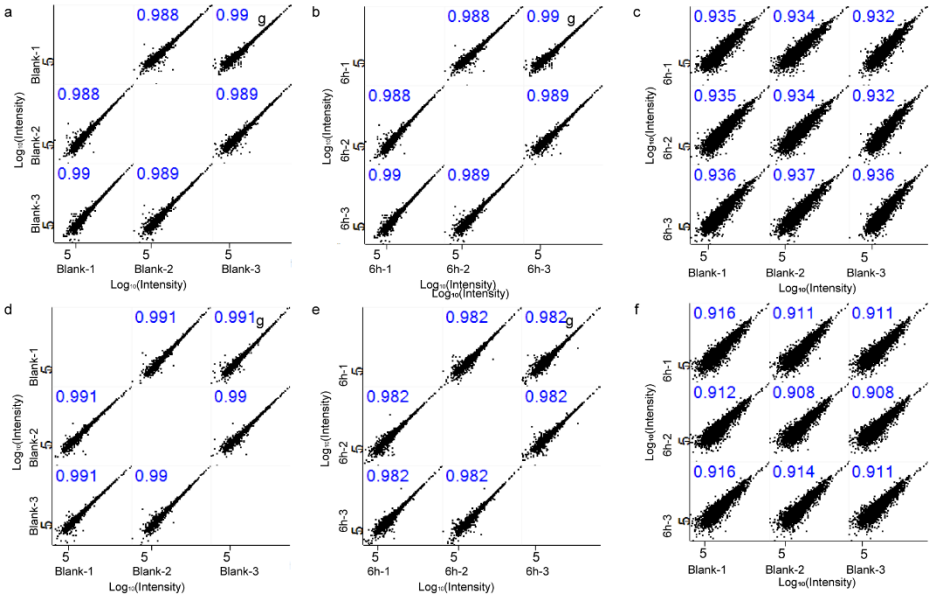
Sample	10DDAB@PLGA/Kolliphor EL NPs	40DDAB@PLGA/Kolliphor EL NPs	70DDAB@PLGA/Kolliphor EL NPs
Loading efficiency (%)	0.0881	19.37	24.84



Supplementary Figure 1: Reproducibility of measurements. a-c Protein intensity measurements of HepG2 cells treated with DDAB@PLGA/Kolliphor EL NPs at 1, 3, and 6 h were plotted against with blank, and correlation is determined between measurements by Pearson's coefficient. d-g Protein intensity measurements of HepG2 cells treated with DDAB@PLGA/Kolliphor EL NPs were plotted against themselves and correlations between measurements were determined by Pearson's coefficient.

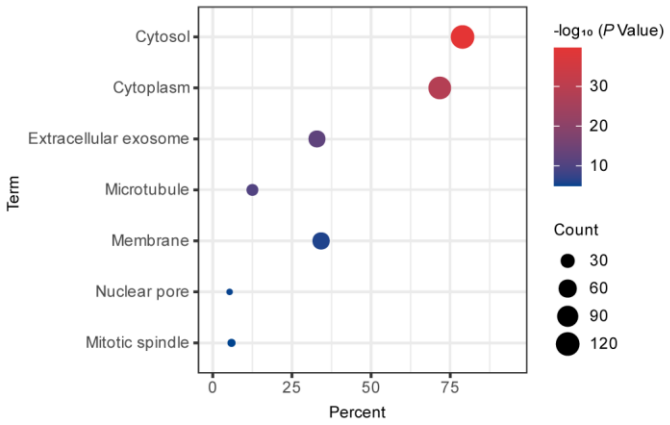


Supplementary Figure 2: Reproducibility of measurements. a-c Protein intensity measurements of HepG2 cells treated with 10DSS-DDAB@PLGA/Kolliphor EL NPs at 1, 3, and 6 h were plotted against with blank, and correlation is determined between measurements by Pearson's coefficient. d-g Protein intensity measurements of HepG2 cells treated with 10DSS-DDAB@PLGA/Kolliphor EL NPs were plotted against themselves and correlations between measurements were determined by Pearson's coefficient.

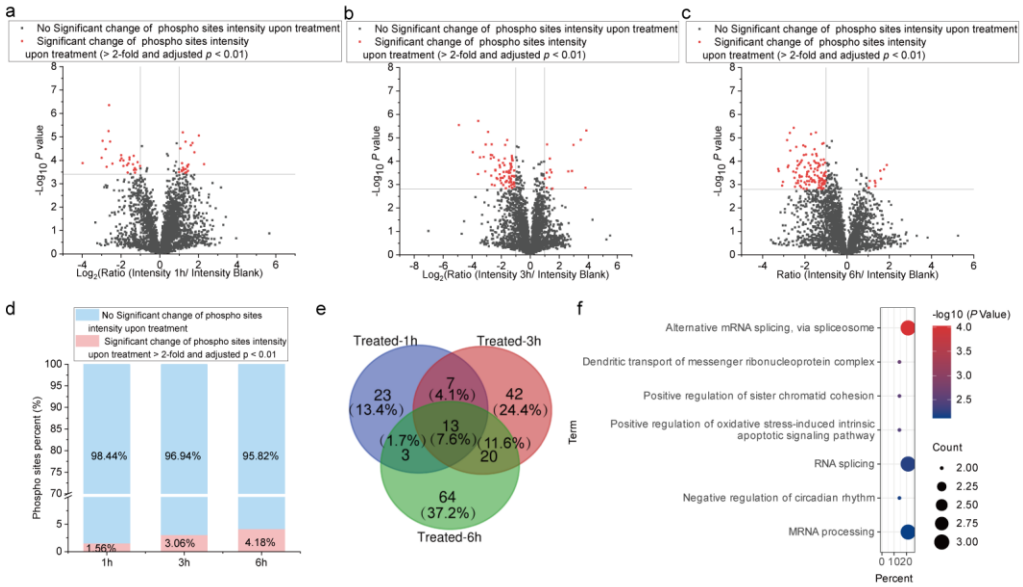


Supplementary Figure 3: Reproducibility of measurements. a-b Protein intensity measurements of HepG2 cells and HepG2 cells treated with 40DSS-DDAB@PLGA/Kolliphor EL NPs for 6 h were plotted against themselves. c Protein intensity measurements from HepG2 cells treated with 40DSS-DDAB@PLGA/Kolliphor EL NPs for 6 h were plotted against blank. d-e Protein intensity measurements of HepG2 cells and HepG2 cells treated with 70DSS-

DDAB@PLGA/Kolliphor EL NPs for 6 h were plotted against themselves. f Protein intensity measurements from HepG2 cells treated with 70DSS-DDAB@PLGA/Kolliphor EL NPs for 6 h were plotted against blank, and the correlation between the measurements was determined by Pearson coefficient.

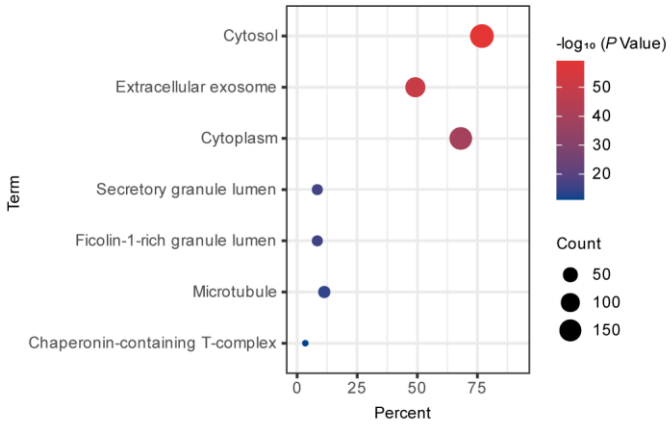


Supplementary Figure 4: GO analysis of significant changed proteins. GO cellular component analysis results of the changed proteins identified in DDAB@PLGA/Kolliphor EL NP-treated HepG2 cells at all three time points.

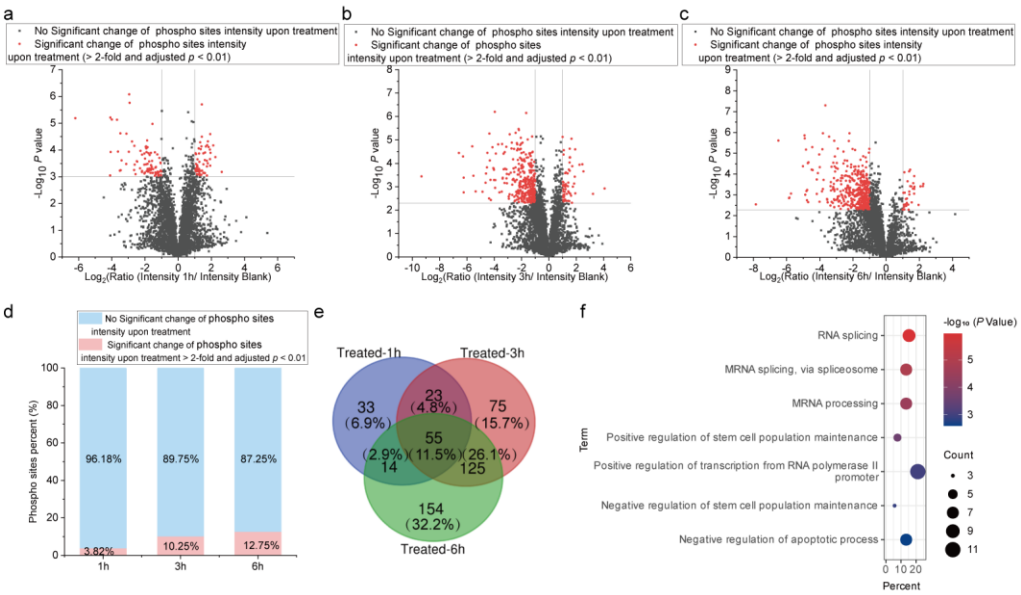


Supplementary Figure 5: Significant changes in phosphorylated protein abundance. a-c Label-free quantification was performed to calculate changes in the abundance of phosphorylated protein sites between native and different DDAB@PLGA/Kolliphor EL NP-treated HepG2 cells for different times. Phosphorylated protein sites with a significant change in intensity of more than 2-fold in the treated group compared to the untreated group are shown as red dots, while the remaining proteins are shown as black dots. d The percentage of phosphorylation site intensities that significantly changed by more than 2-fold in the treated group compared to the untreated group is shown in light pink, while the remaining proteins are shown in sky blue. *P* value was calculated by using Student's T-test (adjusted $p < 0.01$). The P value was adjusted for multiple tests using FDR (Permutation-based). e Venn diagram

of the changed proteins at three time points. f GO analysis results for proteins with changes identified in all three time points were classified by biological process.

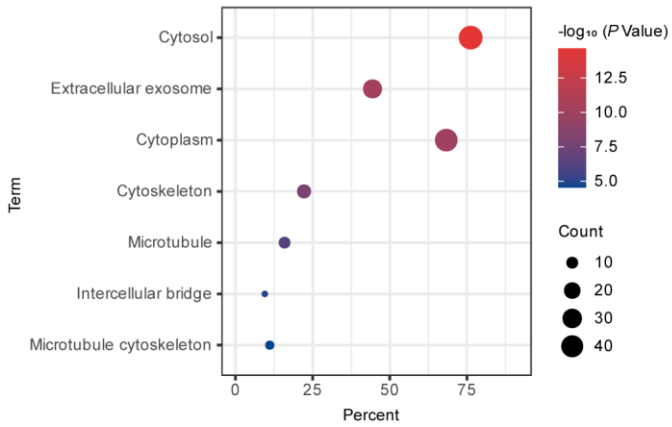


Supplementary Figure 6: GO analysis of significant changed proteins. GO cellular component analysis results of the changed proteins identified in 10DSS-DDAB@PLGA/Kolliphor EL NPs -treated HepG2 cells at all three time points.



Supplementary Figure 7: Significant changes in phosphorylated protein abundance. a-c Label-free quantification was performed to calculate changes in the abundance of phosphorylated protein sites between native and different 10DSS-DDAB@PLGA/Kolliphor EL NPs-treated HepG2 cells for different times. Phosphorylated protein sites with a significant change in intensity of more than 2-fold in the treated group compared to the untreated group are shown as red dots, while the remaining proteins are shown as black dots. d The percentage of phosphorylation site intensities that significantly changed by more than 2-fold in the treated group compared to the untreated group is shown in light pink, while the remaining proteins are shown in sky blue. *P* value was calculated by using Student's T-test (adjusted $p < 0.01$). The P value was adjusted for multiple tests using FDR (Permutation-based). e Venn diagram of the changed proteins at three time points. f GO analysis results for proteins with

changes identified in all three time points were classified by biological process.



Supplementary Figure 8: GO analysis of significant changed proteins. GO cellular component analysis results of the changed proteins identified in different NPs-treated HepG2 cells for 6h. Treated-1 (10DSS-DDAB@PLGA/Kolliphor EL NPs were incubated with HepG2 cells for 6 h), Treated-2 (40DSS-DDAB@PLGA/Kolliphor EL NPs were incubated with HepG2 cells for 6 h) and Treated-3 (70DSS-DDAB@PLGA/Kolliphor EL NPs were incubated with HepG2 cells for 6 h).